Magnesium Ion-Dependent Diagnostic System for Gene Mutation

Mizuo MAEDA, Graduate School of Engineering, Kyushu University
Yoshiki KATAYAMA, Graduate School of Engineering, Kyushu University

It has been found that small mutations of certain genes are the definitive origin of many heritable disorders and cancers. Such new findings have highlighted the importance of gene mutation assays based on the difference of DNA base sequences in diagnostic or medical field. Capillary electrophoresis can be a good candidate for an ideal method on such gene analysis, because the methods can be performed with trace amount of samples, high resolution and shorter running time. We describe here an effect of oligonucleotide, which was introduced in poly(acrylamide) as a branch, on the recognition of an overall sequence of sample DNA fragments. This method possesses real potential for the gene mutation analysis.

We studied the affinity capillary electrophoresis using $(dT)_{12}$ as an affinity ligand. $(dT)_{12}$ was immobilized on poly(acrylamide). If magnesium ion was not added, every oligonucleotide was detected in very similar manner to the case of simple polyacrylamide coated capillary. In contrast, the addition of $MgCl_2$ brought about a dramatic effect in the detection of $(dA)_{12}$ which is complementary partner of the immobilized $(dT)_{12}$. The peak of $(dA)_{12}$ was gradually retarded with increasing concentration of Mg^{2+}, and finally separeted from all other nucleotides havinng one base mismatch. The peak retardation of $(dA)_{12}$ in high Mg^{2+} concentration would be due to the enhancement of affinity of $(dA)_{12}$ with the immobilized $(dT)_{12}$. Mg^{2+} is known to stabilize a DNA duplex, making tight complex with anionic phosphates in the DNA strand.

To generalize this affinity capillary electrophoresis, we applied this system to an analysis of K-ras sequence and its one base mutant. Ras protein, which is a family of small G-proten, is very important in a cellular signal transduction for cell proliferation or differentiation.. It is also reported that a point mutation at a certain base on codon 12 in K-ras gene is one of the major origin of cancer. Thus, anti-sense sequence of c-K-ras codon 11-12 (5'-ACCAGC-3') was immobilized on poly(acrylamide) chain. The results were very similar to those of analyses of $(dA)_{12}$ and its one base mutant sequences using the $(dT)_{12}$ carrying poly(acrylamide). Detection peak for c-K-ras codon 10-13 (5'-GGAGCTGGC-3'), which is complementary partner of the affinity ligand, again gradually retarded as increasing Mg^{2+} concentration, while the one base mutant having the sequence of 5'-GGAGCTAGTGGC-3' was clearly separated from the wild type in the presence of Mg^{2+} at a concentration of 250 μM..

In this system, only the peak for the DNA fragment, which has perfect complementary sequence to the immobilized ligand, should be retarded with controlling the Mg^{2+} concentration. Thus, the mutation of certain gene would be simply determined by the base-line separation of peaks under an appropriate Mg^{2+} concentration. This method would allow quantitative determination of normal and mutant genes at the same time on-line.